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<b>(21) International Application Number:</b> PCT/NZ99/00003 <b>(22) International Filing Date:</b> 14 January 1999 (14.01.99)  <b>(30) Priority Data:</b> 329582 14 January 1998 (14.01.98) NZ  <b>(71) Applicant (for all designated States except US):</b> THE CORPORATION OF THE TRUSTEES OF THE ORDER OF THE SISTERS OF MERCY IN QUEENSLAND [AU/AU]; Raymond Terrace, South Brisbane, QLD (AU).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> HART, Derek, Nigel, John [NZ/AU]; 3 Borva Street, Dutton Park, Brisbane, QLD 4102 (AU).  <b>(74) Agents:</b> BENNETT, Michael, Roy et al.; Russell McVeagh West-Walker, The Todd Building, Level 5, 171-177 Lambton Quay, Wellington 6001 (NZ).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> HUMAN CMRF-35-H9 RECEPTOR WHICH BINDS IgM  <b>(57) Abstract</b> <p>The invention relates to the receptor CMRF-35-H9 which <i>inter alia</i> bind immunoglobulin M (IgM). The receptor, together with peptides encoding its extracellular domain, have application in methods of immunomodulation, including in the blocking or inhibition of humoral immune responses in transplant patients.</p> <div data-bbox="854 1493 1354 1591" style="border: 1px solid black; padding: 10px; margin-top: 20px;"><b>REFERENCE: AR</b> C.H. ZLOT, et al., USSN: 09/713,098 Atty. Docket No.: DX01051Q</div>		

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In a further aspect, the invention provides a peptide encoding domain of receptor CMRF-35-H9, which is comprised of amino acids 14 to 177 of the amino acid sequence of Figure 2 (SEQ ID NO. 3), or a functionally equivalent variant thereof.

- 5 In a still further aspect, the invention provides a polynucleotide encoding receptor CMRF-35-H9 and/or its extracellular domain as defined above. This polynucleotide molecule is preferably DNA, more preferably cDNA, but can also be RNA.

- 10 In one embodiment, the DNA molecule coding for receptor CMRF-35-H9 comprises the nucleotide sequence set out in Figure 2 (SEQ ID NO. 4), or a sequence which is a functionally equivalent variant thereof.

- 15 In a further embodiment, the present invention provides a DNA molecule coding for a peptide encoding the extracellular domain of human CMRF-35-H9 which comprises nucleotides 120 to 611 of Figure 2 (SEQ ID NO. 6).

In yet a further aspect, the invention provides a vector including a polynucleotide as defined above.

- 20 In still a further aspect, the invention provides a method of producing receptor CMRF-35-H9 or the extracellular domain thereof comprising the steps of:
- (a) culturing a suitable host cell which has been transformed or transfected with a vector as defined above to express the encoded receptor CMRF-35-H9 or extracellular domain; and
  - 25 (b) recovering the expressed receptor CMRF-35-H9 or extracellular domain.

- 30 In a still further aspect, the present invention provides for the use of receptor CMRF-35-H9 or extracellular domain thereof in the preparation of a medicament suitable for use in methods of therapy or prophylaxis.

Pharmaceutical compositions comprising receptor CMRF-35-H9 or the extracellular domain thereof also form part of the present invention.

## HUMAN CMRF-35-H9 RECEPTOR WHICH BINDS IgM

### FIELD OF THE INVENTION

- 5 This invention relates to the receptor CMRF-35-H9 which *inter alia* binds immunoglobulin M(IgM)

### BACKGROUND OF THE INVENTION

- 10 It is well known that the human immune response is stimulated by foreign antigen (Ag). Antigen presenting cells, such as the dendritic cells perform important immunoregulatory functions by presenting antigens in the form of peptides bound to cell-surface major histocompatibility complex (MHC) molecules to T cells. This initiates a T lymphocyte response which is followed by a humoral or antibody (B  
15 lymphocyte derived) immune response. Humoral responses include a primary response with antibodies of the IgM isotype followed by a secondary response with immunoglobulin of the IgG, IgA and IgE isotype. The soluble immunoglobulin interact with Ag in the tissues (opsonisation) and bind, via their functional components (Fc) to receptors (Fc receptors) on different types of white blood cells.

- 20 Given that IgM is the primary antibody produced, the identification and characterisation of cellular IgM receptors and binding proteins has important implications in manipulating immune response in prophylaxis and therapy, particularly in humans.

- 25 The applicant has now identified a receptor on human dendritic and other cells which binds IgM. It is broadly to this receptor, which the applicants have called CMRF-35-H9, that the present invention is directed.

### 30 SUMMARY OF THE INVENTION

- The present invention has a number of aspects. In a first aspect, the invention provides human CMRF-35-H9 which has the amino acid sequence set out in Figure 2 (SEQ ID NO. 1), or a functionally equivalent variant thereof.

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Other aspects of the invention will be apparent from the description which follows and from the attached claims.

### DESCRIPTION OF THE DRAWINGS

5

While the invention is broadly as defined above, it will be appreciated by those persons skilled in this art that it is not limited thereto and that it includes embodiments more particularly described below.

10 In particular, preferred aspects of the invention will be described in relation to the accompanying drawings in which:

Figure 1 represents the structure of the receptor of the invention wherein:

A is a 13 amino acid hydrophobic leader sequence;

15

B is a 164 amino acid extracellular domain;

C is a 24 amino acid transmembrane region; and

D is a 100 amino acid cytoplasmic domain.

Figure 2 represents both the amino acid sequence of the CMRF-35-H9 receptor of the invention and the nucleotides coding therefor. The leader sequence and transmembrane region are underlined. The putative IgM binding domain within the extracellular domain is shown in square brackets. "N" in the sequence indicates an undetermined nucleotide.

25

Figure 3 depicts L cells transfected with the CMRF-35 cDNA binding the CMRF-35 mAb. Panel A shows reactivity of the CMRF-35 mAb with the parental L cells. Panel B is the histogram for the CMRF-35 mAb reactivity with the L cell transfectants expressing CMRF-35 cDNA.

30

Figure 4 depicts binding of mouse immunoglobulin to the L cell transfectants. The histograms on the left of the diagram shows the reactivity of mouse immunoglobulins to L cells. On the right are the histograms for the reactivity of mouse immunoglobulins to the CMRF-35

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expressing transfectants. A - no primary antibody, B - IgG1, C - IgG2b, D - IgM, E - IgG1 and F - IgG2b.

- Figure 5 depicts Jurkat cells transfected either with CMRF-35-H9 cDNAs expressing epitopes reacting with the CMRF-35 mAb. A single line indicates Jurkat cells transfected with vector alone.
- Figure 6 depicted are transfected lines rosetting with human RBC coated with mouse antibodies of the IgM isotype (Figure 6a) but not IgG2b isotypes (Figure 6b).
- Figure 7 the CMRF-35-Ig fusion protein is depicted binding mouse IgM but not mouse IgG2a or IgG1.
- Figure 8 is an autoradiograph of cell lines transfected with CMRF-35-H9, CMRF-35 and vector only, as well as untransfected cell lines, when reacted with IgM isotype mAb CMRF-75.

## DESCRIPTION OF THE INVENTION

20

### A. Human CMRF-35-H9

Human CMRF-35-H9 of the invention is a new member of the immunoglobulin (Ig) gene superfamily. More importantly, CMRF-35-H9 binds IgM. The general structure for the receptor is given in Figure 1. The receptor has the amino acid and nucleotide sequences shown in Figure 2.

- 5 -

Individual amino acids are represented by the single letter code as follows:

5	Amino Acid	Three-letter abbreviation	One-letter symbol
	Alanine	Ala	A
	Arginine	Arg	R
10	Asparagine	Asn	N
	Aspartic acid	Asp	D
	Asparagine or aspartic acid	Asx	B
	Cysteine	Cys	C
	Glutamine	Gln	Q
15	Glutamic Acid	Glu	E
	Glutamine or glutamic acid	Glx	Z
	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
20	Leucine	Leu	L
	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
25	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V
30			

The amino acid sequence includes a 13 amino acid leader sequence, a 164 amino acid extracellular domain, a 24 amino acid transmembrane region and a 100 amino acid cytoplasmic region.

35

The extracellular domain further includes an Ig binding domain approximately from amino acids 29 to 126 of Figure 2. This domain includes two sites for N-linked glycosylation between amino acids 82 to 84 and 91 to 93.

40

The membrane proximal region of 45 amino acids contains a number of serine and threonine residues suggesting that this region may contain some O-linked glycosylation. In addition, the region contains proline residues indicating that this hinge region may form a fairly rigid structure.

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The 100 amino acid cytoplasmic region of human CMRF-35-H9 contains four tyrosine-containing motifs. By analogy with similar motifs in other transmembrane regions this suggests that this molecule may have a signal transduction role, either positively or negatively.

5

Human CMRF-35-H9 can usefully be provided in a number of different forms. These include human CMRF-35-H9 itself, the "mature" form of human CMRF-35-H9, and the extracellular receptor domain of human CMRF-35-H9.

10 The "mature" form of human CMRF-35-H9 of the invention is human CMRF-35-H9 less its native amino-terminus leader or signal sequence, whereas the extracellular receptor domain is human CMRF-35-H9 lacking both the transmembrane region and cytoplasmic domain (where present).

15 The invention is of course not restricted to receptors having the specific sequences of Figure 2. Functionally equivalent variants are also contemplated.

The phrase "functionally equivalent variants" recognises that it is possible to vary the amino acid sequence of a protein while retaining substantially equivalent  
20 functionality. For example, a protein can be considered a functional equivalent of another protein for a specific function if the equivalent protein is immunologically cross-reactive with and has at least substantially the same function as the original protein. The equivalent can be, for example, a fragment of the protein, a smaller-sized version of the protein from which one or more amino acids (such as amino  
25 acids 210 to 212 of the Figure 2 sequence) have been deleted (resulting in SEQ ID NO. 2), a fusion of the protein with another protein or carrier, or a fusion of the protein or of a fragment with additional amino acids. It is also possible to substitute amino acids in a sequence with equivalent amino acids using conventional techniques. Groups of amino acids normally held to be equivalent are:

30

- (a) Ala, Ser, Thr, Pro, Gly;
- (b) Asn, Asp, Glu, Gln;
- (c) His, Arg, Lys;
- (d) Met, Leu, Ile, Val; and
- 35 (e) Phe, Tyr, Trp.



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Homologs to human CMRF-35-H9 in other mammals are also "functionally equivalent variants" in the sense this phrase is used herein.

- 5 The probability of one amino acid sequence being functionally equivalent to another can be measured by the computer algorithms BLASTP (Altschul *et al* 1990 J Mol Biol 215:403-410).

Collectively, all of the above constitute "receptor CMRF-35-H9".

10

Receptor CMRF-35-H9 of the invention or its extracellular receptor domains may be prepared by methods known in the art. Such methods include protein synthesis from individual amino acids as described by Stuart and Young in "Solid Phase Peptide Synthesis", Second Edition, Pierce Chemical Company (1984). It is however  
15 preferred that human CMRF-35-H9 and/or its extracellular receptor domain be prepared by recombinant methods.

B. Polynucleotides Encoding Receptor CMRF-35-H9

- 20 In another aspect of this invention, the applicants provide polynucleotides encoding receptor CMRF-35-H9 or its extracellular domain. These polynucleotides may be DNA (isolated from nature, synthesised or cDNA) or RNA. Most often, the polynucleotides will be cDNA.

- 25 In one embodiment, the polynucleotide of the invention comprises the nucleotides encoded by the sequence of Figure 2, or the coding region thereof between substantially nucleotides 81 to 986. In a further embodiment the polynucleotide comprises the molecule encoded by nucleotides 120 to 611.

- 30 Again, the invention is not restricted to polynucleotides having the specific sequence of Figure 2. Functionally equivalent variants of the Figure 2 sequence are also contemplated, including the polynucleotide having the sequence of SEQ ID NO. 5.

The phrase "functionally equivalent variants" recognises that it is possible to vary the nucleotide sequence coding for a protein and to still express either the same

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protein (having the same amino acid sequence due to the degeneracy of the nucleic acid code) or a protein having equivalent functionality.

5 The probability of one nucleic acid nucleotide being functionally equivalent to another can be measured by the computer algorithm, including by FASTA (Pearson *et al* 1988 *Proc. Natl. Acad. Sci.* 85 2444-2448).

10 Nucleotide sequences coding for homologs to human CMRF-35-H9 in other mammals are also contemplated as "functionally equivalent variants" as this term is used herein.

### C. Recombinant Expression of Human CMRF-35-H9

In yet another aspect, the present invention relates to the recombinant expression of receptor CMRF-35-H9 or its extracellular domain.

15

The polynucleotides that encode CMRF-35-H9 or the extracellular domain may be inserted into known vectors for use in standard recombinant DNA expression protocols. Standard recombinant techniques are those such as are described in Sambrook *et al.*; "Molecular Cloning" 2nd Edition Cold Spring Harbour Laboratory Press (1987) and by Ausubel *et al.*, Eds, "Current Protocols in Molecular Biology" 20 Greene Publishing Associates and Wiley-Interscience, New York (1987).

Vectors useful in eucaryotes such as yeast are available and well known. A suitable example is the 2m plasmid.

25

Suitable vectors for use in mammalian cells are also known. Such vectors include pcDNA3 (Invitrogen) well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and vectors derived from combination of plasmids and phage DNA.

30 A presently preferred mammalian cell expression vector is pcDNA3 (Invitrogen).

Further eucaryotic expression vectors are known in the art (e.g. P.J. Southern and P. Berg, *J. Mol. Appl. Genet.* 1: 327-341 (1982); S. Subramani *et al.*, *Mol. Cell. Biol.* 1: 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "Amplification And Expression of 35 Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary

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- 10 The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g. the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g. Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from cytomegalovirus (CMV), polyoma, adenovirus, retrovirus, and simian virus, e.g. the early and late promoters or SV40, and other sequences known to control the expression of genes of eucaryotic cells and their viruses or combinations thereof.

A presently preferred promoter is a CMV promoter.

- 25 In the construction of a vector it is also an advantage to be able to distinguish the vector incorporating the foreign DNA from unmodified vectors by a convenient and rapid assay. Such assays include measurable colour changes, antibiotic resistance and the like. In one preferred vector, the  $\beta$ -galactosidase gene is used, which gene is detectable by clones exhibiting a blue phenotype on X-gel plates. This facilitates selection. Once selected, the vectors may be isolated from the culture using routine procedures such as freeze-thaw extraction followed by purification.

Vectors containing the receptor-encoding DNA and control signals are inserted into a host cell for expression of the receptor. Some useful expression host cells include well-known eucaryotic cells. Suitable eucaryotic cells include yeast and other fungi,

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insect, animal cells, such as mouse L cells (a fibroblast line) COS cells and CHO cells, human cells and plant cells in tissue culture.

Depending on the host used, transformation is performed according to standard techniques appropriate to such cells. For mammalian cells the calcium phosphate precipitation method of Graeme and Van Der Eb, *Virology* 52:546 (1978) is preferred. Transformations into plants may be carried out using Agrobacterium tumefaciens (Shaw et al., *Gene* 23:315 (1983) or into yeast according to the method of Van Solingen et al. *J.Bact.* 130: 946 (1977) and Hsiao et al. *Proceedings, National Academy of Science*, 76: 3829 (1979).

Upon transformation of the selected host with an appropriate vector the polypeptide or peptide encoded can be produced, often in the form of fusion protein, by culturing the host cells. The polypeptide or peptide of the invention may be detected by rapid assays as indicated above. The polypeptide or peptide is then recovered and purified as necessary. Recovery and purification can be achieved using any of those procedures known in the art, for example by adsorption onto and elution from an anion exchange resin.

#### 20 D. Ligands

Ligands that bind to receptor CMRF-35-H9 also have utility.

The ligand will usually be an antibody or an antibody binding fragment raised against receptor CMRF-35-H9 or its extracellular domain.

25

Such antibodies may be polyclonal but are preferably monoclonal. Monoclonal antibodies may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein in *Nature* 256: 495-497 (1975) and Campbell in "Monoclonal Antibody Technology, the Production and Characterization of Rodent and Human Hybridomas" in Burdon et al. Eds, Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA method described by Huse et al. in *Science* 246: 1275-1281 (1989).

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E. Ligand-Antigen constructs

Ligands which bind to receptor CMRF-35-H9 as expressed on antigen-presenting cells (usually antibodies or antibody-binding fragments) can be coupled or otherwise associated with antigens against which an immune response is desired. In use, the  
5 ligand component binds to receptor CMRF-35-H9 and the antigen-presenting cell is 'primed' with the associated antigen. This 'priming' action will assist in the induction of an immediate immune response against the antigen.

The ligand-antigen construct can take any appropriate form for administration to  
10 the antigen-presenting cells. Such forms may differ depending upon whether the therapeutic protocol involves isolation of the patients antigen-presenting cells (so that the priming action can take place *in vitro*) or whether the construct is to be administered to a patient *in vivo*.

15 One example of a construct for administration to a patient *in vivo* is a live recombinant viral vaccine. Such a vaccine includes nucleic acid encoding the CMRF-35-H9 ligand (or a portion thereof) and the antigen. The vaccine is administered to the patient and, once within the patient, expresses the encoded ligand and antigen to bind to the patients antigen-presenting cells (via receptor  
20 CMRF-35-H9).

A number of such live recombinant viral vaccine systems are known. An example of such a system is the *Vaccinia* virus system (US Patent 4603112; Brochier et al., *Nature* 354: 520 (1991)).

25

**EXPERIMENTAL**

Various aspects of the invention will now be described with reference to the following experiments which demonstrate that receptor CMRF-35-H9 is a molecule  
30 which is distinct from CMRF-35, and that CMRF-35-H9 binds IgM.

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A. Receptor activity of CMRF-35-H9Materials and methods

5 Antibodies: The following antibodies were used; CMRF-35 (IgG<sub>2a</sub>) (Daish *et al.*, Immunology 79:55-63 (1993)), Sal5 (IgG<sub>2a</sub> isotype control, obtained from Professor H Zola, Adelaide). Flurosecein isothiocyanate-conjugated goat anti-human Fab fragments specific for human IgG, IgA and IgM chains were obtained from DAKO. The following antibodies were produced in the laboratory using standard  
10 techniques; CMRF-7 (CD15, IgM), CMRF-10 (erythrocyte b sialoglycoprotein, IgG<sub>1</sub>), CMRF14 (erythrocyte a sialoglycoprotein, IgG<sub>2b</sub>), CMRF-15 (erythrocyte a sialoglycoprotein, IgM), CMRF-17 (B lymphocyte activation antigen, IgM).

Immunoglobulins: Purified IgM, IgG and IgA were bought from DAKO.

15

Purification of IgM: Human IgM was purified from human plasma by dialysis O/N against H<sub>2</sub>O to precipitate the euglobulin. The euglobulin was dissolved in 50mM Tris ClpHSO, 10mM CaCl<sub>2</sub> and passed over a Sephacryl 300 column. The void eluate was run on a 4-35% gradient gel under reducing conditions to assess the  
20 purity of IgM.

Digestion of IgM: Fcm5 were prepared by digestion of IgM with trypsin at 60°C and purified on a Sephacryl 300 column.

25 Aggregation of IgM and IgG: Purified IgM (10-20mg/ml) was incubated at 63°C for 30 minutes in glass tubes. The protein was cooled on ice and dilute with PBS pH 8.0 before pelleting the precipitate by centrifugation at 145,000g for 60 min at RT. The pellet was resuspended in PBS pH 8.0 and used at approximately 2.5mg/ml protein.

30 Cells: All cells were grown and maintained in RPMI media supplemented with 10% FCS, glutamine, penicillin and streptomycin.

Transfectants: The mouse L cell fibroblast line was transfected with an expression construct for the CMRF-35 cDNA in the pcDNA2 (Invitrogen) expression vector.

35 Stable transfectants were selected after electroporation. Even with Geneticin

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selection the levels of cell surface CMRF-35 decreased over time and after preliminary experiments these transfectants were abandoned in favour of haemopoietic cell based transfectants. Jurkat cells were transfected by electroporation with the cDNAs for CMRF-35 and CMRF-35-H9 in the mammalian expression vector pcDNA3 (Invitrogen). Transfected cells were selected with 600mg/ml Geneticin (Life Technologies) and maintained with 200mg/ml Geneticin. Routine flow cytometry was used to monitor expression.

Flow cytometry: Cells ( $0.5 \times 10^5$ ) were incubated on ice with saturating amounts of antibody or immunoglobulin (100mg/ml for 30 mins, washed twice in PBS/1% BSA and then labelled with specific FITC conjugated antibody. The primary labelled cells were either analysed on the FACS Vantage or double labelled with phycoerythrin conjugated antibodies before analysis. For double labelling, the cells were washed as before and then incubated in 10% normal mouse serum for 10 mins on ice. The cells were then labelled with directly conjugated antibodies for 30 mins on ice, washed and then analysed.

Rosetting: Human A-RBC were washed in normal saline and resuspended at a 5% solution. An equal volume (25ml) 5% RBC was incubated with (25ml) mAb or human serum for 30 mins at 37°C. The coated cells were then washed in PBS and resuspended at 2% solution. The mAb were used at saturating concentrations and the human serum at a predetermined dilution that resulted in limited aggregation. The coated RBC were then incubated with the transfected cells for 5 mins at 37°C, pelleted at 800rpm for 3 mins and then incubated on ice for 30 mins. The cells were stained with 1% ethyl violet and rosettes were visualised by light microscopy.

Alternatively, human serum was replaced with mouse mAb that recognised epitopes on human RBC. In particular, the following mAb were used; CMRF-7 (CD15, IgM), CMRF-10 (erythrocyte b sialoglycoprotein, IgG<sub>1</sub>), CMRF14 (erythrocyte a sialoglycoprotein, IgG<sub>2b</sub>), CMRF-15 (erythrocyte a sialoglycoprotein, IgM), CMRF-17 (B lymphocyte activation antigen, IgM).

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## Results

### (i) Binding of the CMRF-35 cDNA Expressed in L Cells to Mouse Ig

Expression cloning was used to isolate a cDNA that encoded the epitope for the CMRF-35 mAb. This translated sequence of this cDNA indicated that the protein  
5 had a single V-like domain indicating that it was a member of the Ig superfamily. It was most similar to the Ig binding domains (domain V1 and V4) of the Ig receptor for polymeric IgA and IgM.

To determine if the CMRF-35 cDNA expressed a molecule that bound a form of  
10 immunoglobulin, stable transfectants were made. Initially, these were made in the mouse fibroblast cell line, L cells. Figure 3 shows that these cells expressed a cell surface molecule that bound to the CMRF-35 mAb compared to the parental L cells. Initial experiments were performed to determine if these transfectants expressed well characterised receptors for IgG. The transfectants did not bind mAb for specific  
15 CD16, CD32 or CD64 (Figure 4). However they did appear to specifically bind mouse mAb with the IgM and IgG2<sub>b</sub> isotypes. This indicated that the CMRF-35 cell surface molecule bound the Fc portion of these two isotypes when the immunoglobulin was in a monomeric form.

### 20 (ii) Rosetting of the CMRF-35 cDNA Expressing L Cell Transfectants

Rosetting analysis was performed to determine if the binding of antibody to specific antigen resulted in conformational changes to the Fc portions of the immunoglobulin. Rosettes were formed with L cell transfectants expressing CMRF-35 and RBC coated with antibodies of the IgM isotypes but not with RBC coated  
25 with IgG2b (Figure 6).

### (iii) Expression of Both cDNAs in Haemopoietic Cells, Jurkat

In subsequent analysis a second cDNA product was identified that encodes an epitope recognised by the CMRF-35 mAb, CMRF-35-H9 cDNA (Figure 1).  
30 Transfectants expressing the CMRF-35 cDNA or the CMRF-35-H9 cDNA were made in the acute T lymphocyte leukaemic line, Jurkat. Stable transfectants were selected with Geneticin. Transfectants expressing either cDNA bound the CMRF-35 mAb as detected by flow cytometry (Figure 5).



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(iv) Binding of Human Immunoglobulins to the Transfectants

Monomeric IgG, IgA and IgM was incubated with the transfectants and binding effected using the second stage anti-human antibodies. Figure 4 shows the flow cytometry profiles for the binding of the immunoglobulins to the CMRF-35-H9  
5 expressing transfectants. CMRF-35-Ig bound mouse IgM but not mouse Ig2a or IgG1.

Rosetting of the CMRF-35-H9 and vector only transfectants with human RBC coated with either mouse mAb or human serum were used to determine if the CMRF-35-H9  
10 molecule was able to bind IgM. Binding of the coated RBC with the transfectants was observed.

Conclusion

15 Receptor CMRF-H9 binds IgM and is a distinct molecule from CMRF-35, notwithstanding that both are bound by mAb CMRF-35.

B. IgM mAb reactivity of cell lines transfected with CMRF-35-H9

Cell lines transfected with CMRF-35-H9, CMRF-35, vector only were subjected to  
20 the following procedures (untransfected cell lines being included as a control):

Cell lysis

Cells at  $10^8$ /ml were solubilised by incubation (1hr, 4°C) of cells in lysis buffer (150mM NaCl, 100mM Tris, 0.02% NaN<sub>3</sub>, pH7.8) containing detergent (0.5% Triton-  
25 X-100 and 0.25% CHAPS) and the protease inhibitor mix, Complete™ (Boehringer Mannheim). Following centrifugation (10,000xg, 10min), lysates (1x10<sup>6</sup> cell equivalents/lane) were fractionated on a reducing SDS-PAGE gel and transferred to nitrocellulose (HybondC, Amersham)

30 Western Blot Detection

Western blot detection was performed on transfected cell lines as follows. Antigens bound to nitrocellulose membranes were renatured by overnight incubation at 37°C in 100-200ml PBS with gentle shaking. Membranes were blocked in 5% milk powder/PBS (1hr, room temperature) before incubation (overnight, 4°C) with mAb  
35 solution. For incubation with IgM isotype antibodies CMRF-75 20% human serum

- 16 -

was added to the blocking solution. Purified mAb was used at approximately 10µg/ml in 1% non fat milk powder (MP)/PBS solution and culture supernatant was diluted 4:1 with 5% MP/PBS and supplemented with HEPES (pH7.4) to a final concentration of 10mM. Once again for CMRF-75 antibodies of IgM isotype 20% human serum was added to the diluent. Following incubation, membranes were washed in cold PBS (5x over 5 min) then crosslinked by incubating for 15 min with 0.25% glutaraldehyde/PBS. Membranes were then washed sequentially (15 min each wash) with; 0.1M glycine in PBS, pH8.5 (two changes used) to block residual glutaraldehyde reactive groups, 0.1% BSA/PBS, 1% MP/PBS and 0.1% goat serum (GS)/PBS. Following washing, the membranes were incubated (1hr) with biotin conjugated goat anti-mouse Ig (Dako) diluted (1:1000) in 10% GS/PBS then washed in PBS (10 min) and briefly in 0.05% Tween 20/PBS. Membranes were then incubated (1hr) with streptavidin conjugated horseradish peroxidase (Dako) diluted (1:1000) in 1% BSA/PBS then washed with 0.05% Tween 20/PBS. Reactive protein bands were then visualised by chemiluminescence using Super Signal (Pierce, Illinois, USA) and exposure to autoradiographic film (XAR-5 Kodak). The molecular weight of visualised bands was determined by comparison with biotinylated molecular weight standards (Biorad).

The results are shown in Figure 8. The IgM isotype antibody CMRF-75 reacted *inter alia* with cell lines transfected with CMRF-35-H9.

### Conclusion

IgM isotype antibodies bind to cell lines expressing receptor CMRF-35-H9.

### I. Utilities

The CMRF-35-H9 receptor and extracellular domain thereof have broad utilities in methods of therapy and prophylaxis. These include:

- i) A method for modulating an immune response in a patient, the method comprising administering to said patient receptor CMRF-35-H9 or extracellular domain thereof or ligand or ligand-antigen construct thereto in an amount effective to modulate an immune response.

- 17 -

The term "modulating" is used herein to refer to stimulating, amplifying, blocking or inhibiting an immune response. The ligand will usually be an antibody or antibody binding fragment raised against receptor CMRF-35-H9 or its extracellular domains.

- 5    ii)    A method for blocking or inhibiting a humoral immune response in a patient, the method comprising administering to said patient receptor CMRF-35-H9 or extracellular domain thereof in an amount effective to bind available IgM.

10    In one application of this method, receptor CMRF-35-H9 or the extracellular domain thereof may be administered to a patient receiving a transplant, in an amount effective to inhibit a humoral immune response in that patient.

15    In broad terms, the administration of the CMRF-35-H9 or extracellular domain causes antibody adsorption thereto. The adsorbed antibody is then no longer free to bind to the transplant, thereby inhibiting the humoral immune response.

20    The receptor domain can be administered intravenously, intramuscularly, subcutaneously, topically, orally, intranasally, rectally or intracerebroventricularly, as appropriate. Preparation of administrable forms of the receptor or domain together with pharmaceutically acceptable diluents, carriers or excipients are well known in the art.

25    General assistance in the preparation of such formulations may be obtained from Remingtons Pharmaceutical Sciences, 16th Edition, Easton: Mac Publishing Company (1980); The National formulary XIV 14th Edition, Washington: American Pharmaceutical Association (1975); and Goodman and Gillmans "The Pharmaceutical basis for Therapeutics", 7th Edition, the contents of which are incorporated herein by reference.

- 30    iii)    A method for loading a protective antigen into an antigen presenting cell, the method comprising combining the antigen presenting cell with CMRF-35-H9 receptor/domain ligand-antigen construct. Preferably, the antigen presenting cell is a B-lymphocyte or dendritic cell, most preferably a dendritic cell.

35

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- iv) A related method for stimulating an immune response comprises loading an antigen into an antigen presenting cell according to the method of paragraph iii). Preferably the immune response is a primary T lymphocyte immune response.

5

- v) A method for diagnosing myeloid leukaemia in a patient, comprising:
- (a) determining the CMRF-35-H9 level in a sample from said patient; and
  - (b) comparing the level to a known standard, an increased level of CMRF-35-H9 or decreased level of promyelocytic leukaemia being diagnostic of leukaemia.

10

The term "standard sample" is used to refer to a sample taken from a comparative animal or human which does not have myeloid leukaemia. A level statistically significant above the standard may be diagnostic of leukaemia.

15

Preferably, the sample is a blood or bone marrow sample. Most preferably, a comparison may be made of CMRF-35-H9 levels on CD34+ cells from bone marrow samples. These cells express the CMRF-35-H9 receptor indicating its utility as an early marker in the detection of myeloid leukaemia.

20

Those persons skilled in the art will of course appreciate that the above description is provided by way of example only and that the invention is not limited thereto.

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**CLAIMS**

1. Receptor CMRF-35-H9 which binds IgM and which has an amino acid sequence as set out in SEQ ID NO. 1, or a functionally equivalent variant thereof.
2. Receptor CMRF-35-H9 which binds IgM and which has the amino acid sequence set out in SEQ ID NO. 1.
3. Receptor CMRF-35-H9 which binds IgM and which has the amino acid sequence of SEQ ID NO. 2.
4. A peptide which codes for the extracellular domain of receptor CMRF-35-H9 which binds IgM and which comprises the amino acid sequence of SEQ ID NO. 3, or a functionally equivalent variant thereof.
5. A peptide according to claim 4 which binds IgM and which comprises the amino acid sequence of SEQ ID No. 3.
6. A polynucleotide which encodes receptor CMRF-35-H9 as defined in claim 1 or claim 2.
7. A polynucleotide which encodes receptor CMRF-35-H9 as defined in claim 3.
8. A polynucleotide which encodes a peptide as claimed in claim 4 or claim 5.
9. A polynucleotide as claimed in claim 6 which is DNA.
10. A polynucleotide as claimed in claim 7 which is DNA.
11. A polynucleotide as claimed in claim 8 which is DNA.
12. A polynucleotide as claimed in claim 9 which comprises the nucleotide sequence of SEQ ID NO. 4, or a sequence which is a functionally equivalent variant thereof.
13. A polynucleotide accordingly to claim 12 which comprises the nucleotide sequence of SEQ ID NO. 4.

- 20 -

14. A polynucleotide as claimed in claim 10 which comprises the nucleotide sequence of SEQ ID NO. 5.
15. A polynucleotide which comprises the nucleotide sequence of SEQ ID NO. 6.
16. A vector which includes a polynucleotide as claimed in any one of claims 9  
5 to 15.
17. A method of producing receptor CMRF-35-H9 or a peptide encoding the extracellular domain thereof comprising the steps of:
- 10 (a) culturing a suitable host cell which has been transformed or transfected with a vector as claimed in claim 16 to express the encoded receptor CMRF-35-H9 or extracellular domain; and
- (b) recovering the expressed product.
- 15 18. The use of receptor CMRF-35-H9 or a peptide encoding the extracellular domain thereof as claimed in any one of claims 1 to 5 in the preparation of a medicament.
19. A pharmaceutical composition comprising receptor CMRF-35-H9 or a peptide encoding the extracellular domain thereof as claimed in any one of  
20 claims 1 to 5.
20. A ligand/antigen construct wherein said ligand binds receptor CMRF-35-H9 or a peptide encoding the extracellular domain thereof as claimed in any one of claims 1 to 5, and wherein said antigen is an antigen to which a host immune response is desirable.
- 25 21. A method of modulating an immune response in a patient, the method comprising administering to said patient receptor CMRF-35-H9 or a peptide encoding the extracellular domain thereof as claimed in any one of claims 1 to 5, or a construct as claimed in claim 20, in an amount effective to modulate an immune response.
- 30 22. A method for blocking or inhibiting a humoral immune response in a patient, the method comprising administering to said patient receptor

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CMRF-35-H9 or a peptide encoding the extracellular domain thereof as claimed in any one of claims 1 to 5 in an amount effective to bind available IgM.

23. A method according to claim 22 wherein said patient is a patient who has or  
5 is about to receive a transplant.

## FIGURE 1

CMRF-35-H9

13	164	24	100
A	B	C	D



cgggggaggcggtgactttccctcggtccaggtagggcctggagctgtgcaagtgcgcg 60  
 M W L P W A L L L I W V P G 14  
 cctgtgctggggaagggaccATGTGGCTGCCTTGGGCTCTGTGCTTCTCTGGGTCCTCAG 120  
 C F A L S K C R T V A G P W E S L S V Q 34  
 GATGTTTTGCTCTGAGCAAATGCAGGACCGTGGCGGGCCCGTGGGATCCCTGAGTGTGC 180  
 C P Y E K E H R T L N K Y W C R P P Q I 54  
 AGTGTCCTATGAGAAGGAACACAGGACCTCAACAAATACTGGTGCAGACCACCACAGA 240  
 F L C D K I V E T K G S A G K R N G R V 74  
 TTTTCTATGTGACAAGATTGTGGAGACCAAAGGGTCAGCAGGAAAAAGGAACGCCGAG 300  
 S I R D S P A N L S F T V T L E N L T E 94  
 TGTCCATCAGGGACAGTCTTGCAAACCTCAGCTTCACAGTGACCCTGGAGAATCTCACAG 360  
 E D A G T Y W C G V D T P W L R D F H D 114  
 AGGAGGATGCAGGCACCTACTGCTGTGGGGTGGATACACCATGGCTCCGAGACTTTCATG 420  
 P V V E V E V S V F P A S T S M T P A S 134  
 ATCCCGTTGTGAGGTTGAGGTGTCCGTGTTCCCGGCATCAACGTCAATGACACCTGCAA 480  
 I T A A K T S T I T T A F P P V S S T T 154  
 GTATCACTGCGGCCAAGACCTCAACAATCACAACCTGCATTTCCACCTGTATCATCCACTA 540  
 L F A V G A T H S A S I Q E E T E E V V 174  
 CCCTGTTTGCAGTGGGTGCCACCCACAGTGCCAGCATCCAGGAGGAACTGAGGAGGTGG 600  
 N S Q L P L L L S L L A L L L L L L V G 194  
 TGAACCTACAGCTCCCGCTGCTCCTCTCCCTGCTGGCATTGTTGCTGCTTCTGTTGGTGG 660  
 A S L L A W R M F Q K W I K W I K A G D 214  
 GGGCCTCCCTGCTAGCCTGGAGGATGTTTCAGAAATGGATCAAAGCTGGTG 720  
 H S E L S Q N P K Q A A T Q S E L H Y A 234  
 ACCATTGAGAGCTGTCCCAGAACCCCAAGCAGGCTGCCACGCAGAGTGAGCTGCACTACG 780  
 N L E L L M W P L Q E K P A P P R E V E 254  
 CAAATCTGGAGCTGCTGTATGTGGCTCTGCAGGAAAAGCCAGCACCACCAAGGGAGGTGG 840  
 V E Y S T V A S P R E E L H Y A S V V F 274  
 AGGTGGAATACAGCACTGTGGCCTCCCCCAGGGAAGAACTTCACTATGCCTCGGTGGTGT 900  
 D S N T N R I A A Q R P R E E E P D S D 294  
 TTGATTCTAACCAACAGGATAGCTGCTCAGAGGCCTCGGGAGGAGGAACCAGATTGAG 960

Figure 2/1

3/10

Y S V I R K T \* 301  
ATTACAGTGTGATAAGGAAGACATaggtcctgcctcgccatcggagctctcatgggcccc 1020  
aggaagtcagggacagctcccttataacctggcccacgtccttctcagcctgccctcgaca 1080  
acagtgaccaacagacaggcagctgggtttccaggccatccctctgttgccatcagcttg 1140  
attggcttccccgagggccagcagggctggggctccggagagcagcaggaagcaactcca 1200  
gccaccagtgcctgtcacctcttcccccttgccccctgcttcatcccagctctgtgtgtg 1260  
gaggacaaagcttcttcctgcgtggctccaggaaaagatgtggctcacgtaggtNgcacc 1320  
tgccaatagctttgtcaatcacagcccataggaacgtctggaattgcttgggagttggg 1380  
gagaactgtcaagaagagtgaagagagtgccaaagcggagatctgttcacctgggtggag 1440  
gggaccactaagatcaagatcaaagattctccccatctcacagacaaggaaactgagNNA 1500  
gagggaggagagaattgctcatggctccagaactggtggcaagtttctctggactcttta 1560  
ggtttatttttaatatgaaatataaaaaacagtttcaaatatcttattgagggagaagtaa 1620  
aaacttatttaaaccccg 1640

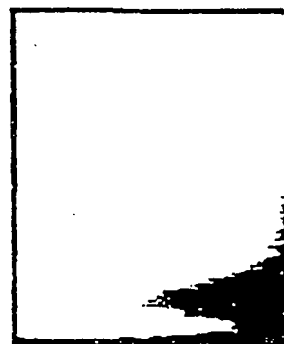
Figure 2/2

EXPRESSION OF CMRF-35 BY L CELL TRANSFECTANTS

CMRF-35  
Transfectants



L Cells



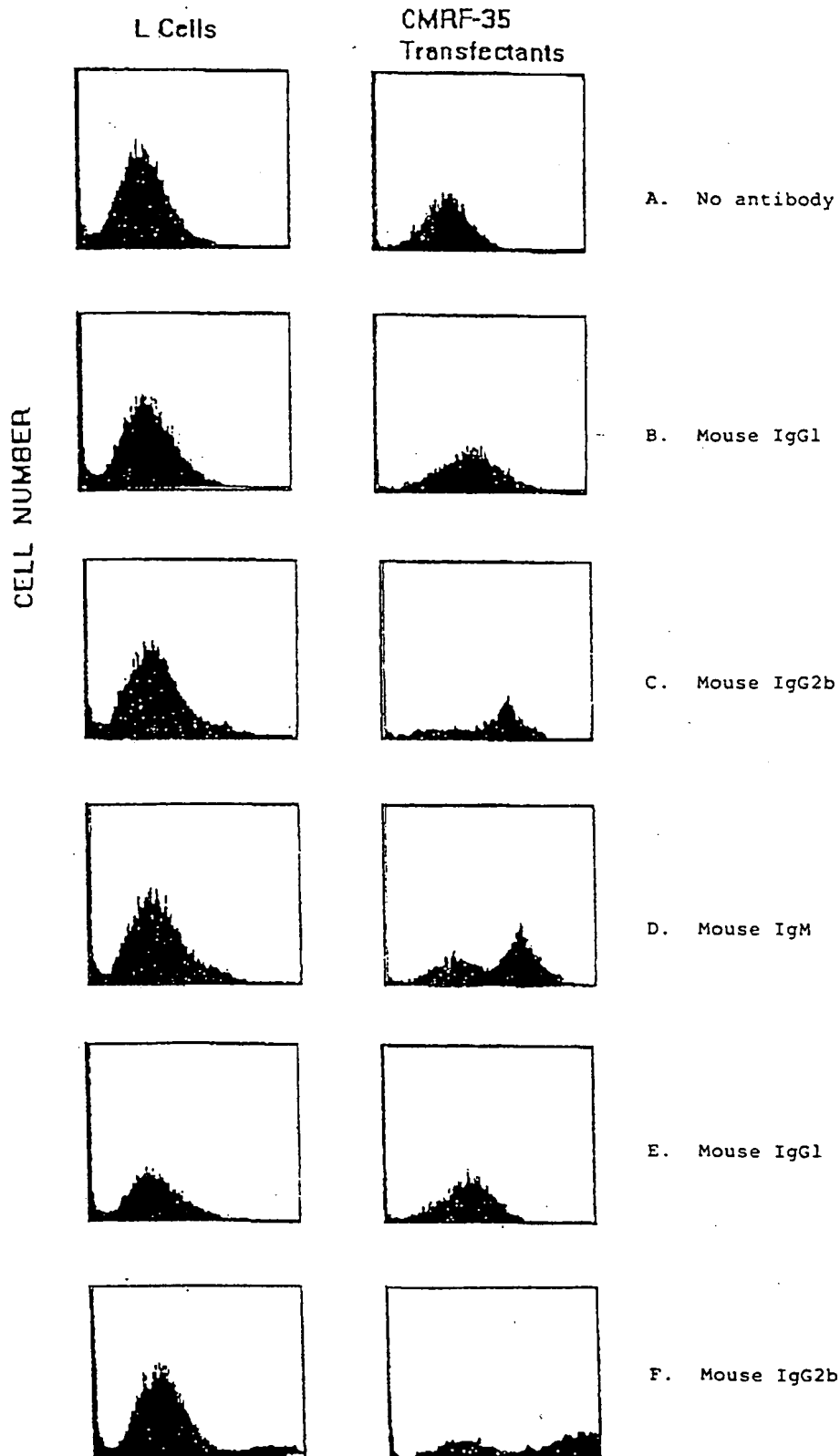
FLUORESCENCE INTENSITY

PANEL B

PANEL A

FIGURE 3

FIGURE 4 5/10



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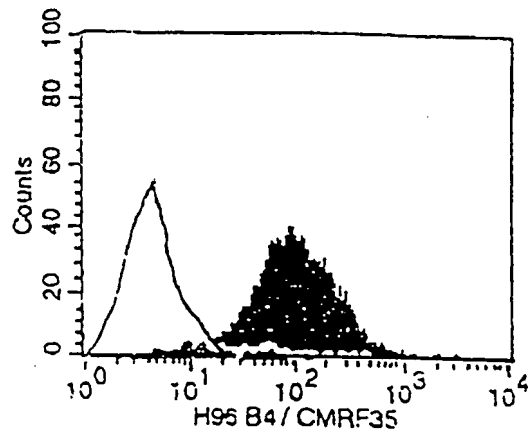


FIGURE 5



FIGURE 6a

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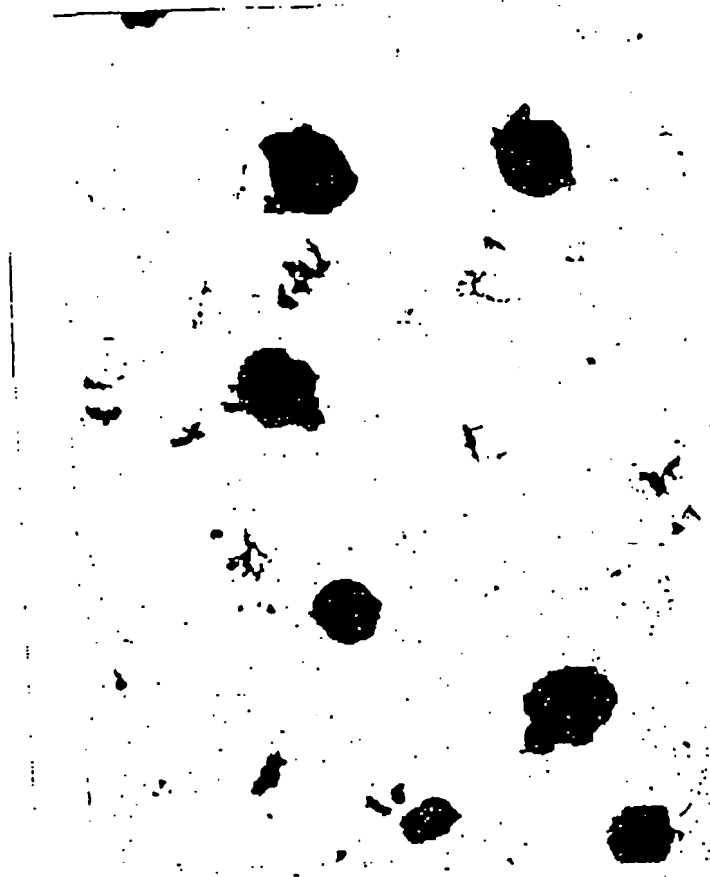


FIGURE 6b

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# Reactivity of mAb with CMRF-35-Ig Fusion Protein

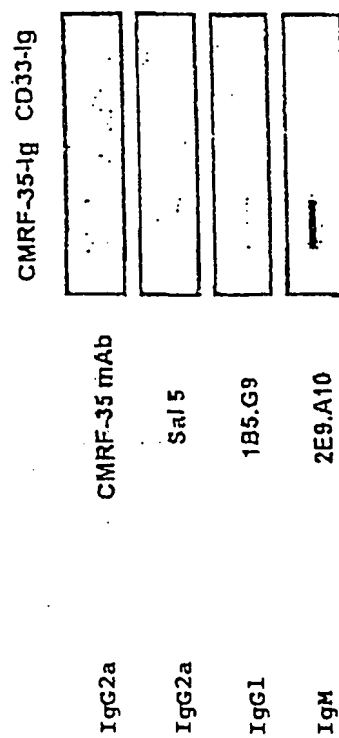


FIGURE 7



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CMRF-75

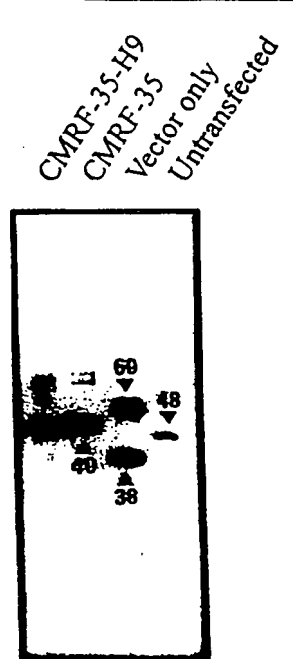


Figure 8

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (1) APPLICANT: THE CORPORATION OF THE TRUSTEES  
OF THE ORDER OF THE SISTERS OF  
MERCY IN QUEENSLAND
- (2) TITLE: RECEPTOR
- (3) NUMBER OF SEQUENCES: 6
- (5) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 3.5"HD FLOPPY DISC
  - (B) COMPUTER: IBM PC COMPATIBLE
  - (C) OPERATING SYSTEM: MS-DOS
  - (D) SOFTWARE: WORD 95

## (2) INFORMATION FOR SEQUENCE ID NO. 1:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 301 AMINO ACIDS
- (B) TYPE: AMINO ACID
- (C) TOPOLOGY: LINEAR

## (2) MOLECULE TYPE: PROTEIN

## (3) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

MWLPWALLLI WVPGCFALSK CRTVAGPWGS LSVQCPYEKE HRTLNKYWCR 50  
PPQIFLCDKI VETKGSAGKR NGRVSIRDSP ANLSFTVTLE NLTEEDAGTY 100  
WCGVDTPWLR DFHDPVVEVE VSVFPASTSM TPASITAAKT STITTAFFPV 150  
SSTTLFAVGA THSASIQEET EEVVNSQLPL LLSLLALLLL LLVGASLLAW 200  
RMFQKWIW KAGDHSELSQ NPKQAATQSE LHYANLELLM WPLQEKPP 250  
REVEVEYSTV ASPREELHYA SVVFDSTNR IAAQRPREEE PDSYSVIRK 300  
T 301

## (2) INFORMATION FOR SEQUENCE ID NO. 2:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 298 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) TOPOLOGY: LINEAR

(2) MOLECULE TYPE: PROTEIN

(3) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

MWLPWALLLI	WVPGCFALSK	CRTVAGPWGS	LSVQCPYEKE	HRTLNKYWCR	50
PPQIFLCDKI	VETKGSAGKR	NGRVSIRDSP	ANLSFTVTLE	NLTEEDAGTY	100
WCGVDTPWLR	DFHDPVVEVE	VSVFPASTSM	TPASITAAKT	STITTAFPPV	150
SSTTLFAVGA	THSASIQEET	EEVNSQLPL	LLSLLALLLL	LLVGASLLAW	200
RMFQKWIKAG	DHSELSQNP	KAATQSELHY	ANLELLMWPL	QEKPAAPPREV	250
EVEYSTVASP	REELHYASVV	FDSNTNRIAA	QRPREEEPDS	DYSVIRKT	298

## (2) INFORMATION FOR SEQUENCE ID NO. 3:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 164 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) TOPOLOGY: LINEAR

(2) MOLECULE TYPE: PROTEIN

(3) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

GCFALSKCRT	VAGPWGSLSV	QCPYEKEHRT	LNKYWCRPPQ	IFLCDKIVET	50
KGSAGKRNGR	VSIRDSPANL	SFTVTLENLT	EEDAGTYWCG	VDTPWLRDFH	100
DPVVEVEVSV	FPASTSMTPA	SITAAKTSTI	TTAFPPVSST	TLFAVGATHS	150
ASIQEETEEV	VNSQ				164

## (2) INFORMATION FOR SEQUENCE ID NO. 4:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 903

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

## (2) MOLECULE TYPE: cDNA

## (3) SEQUENCE DESCRIPTION: SEQ ID NO. 4:

```
ATGTGGCTGC CTTGGGCTCT GTTGCTTCTC TGGGTCCCAG GATGTTTTGC 50
TCTGAGCAAA TGCAGGACCG TGGCGGGCCC GTGGGGATCC CTGAGTGTGC 100
AGTGTCCCTA TGAGAAGGAA CACAGGACCC TCAACAAATA CTGGTGACAGA 150
CCACCACAGA TTTTCCTATG TGACAAGATT GTGGAGACCA AAGGGTCAGC 200
AGGAAAAAGG AACGGCCGAG TGTCCATCAG GGACAGTCCT GCAAACCTCA 250
GCTTCACAGT GACCCTGGAG AATCTCACAG AGGAGGATGC AGGCACCTAC 300
TGGTGTGGGG TGGATACACC ATGGCTCCGA GACTTTCATG ATCCCGTTGT 350
CGAGGTTGAG GTGTCCGTGT TCCCGGCATC AACGTCAATG ACACCTGCAA 400
GTATCACTGC GGCCAAGACC TCAACAATCA CAACTGCATT TCCACCTGTA 450
TCATCCACTA CCCTGTTTGC AGTGGGTGCC ACCCACAGTG CCAGCATCCA 500
GGAGGAAACT GAGGAGGTGG TGAAGTCACA GCTCCCGCTG CTCCTCTCCC 550
TGCTGGCATT GTTGCTGCTT CTGTTGGTGG GGGCCTCCCT GCTAGCCTGG 600
AGGATGTTTC AGAAATGGAT CAAATGGATC AAAGCTGGTG ACCATTCAGA 650
GCTGTCCCAG AACCCCAAGC AGGCTGCCAC GCAGAGTGAG CTGCACTACG 700
CAAATCTGGA GCTGCTGATG TGGCCTCTGC AGGAAAAGCC AGCACCACCA 750
AGGGAGGTGG AGGTGGAATA CAGCACTGTG GCCTCCCCCA GGGAAGAACT 800
TCACTATGCC TCGGTGGTGT TTGATTCTAA CACCAACAGG ATAGCTGCTC 850
AGAGGCCTCG GGAGGAGGAA CCAGATTCAG ATTACAGTGT GATAAGGAAG 900
ACA 903
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## (2) INFORMATION FOR SEQUENCE ID NO. 5:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 894

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(2) MOLECULE TYPE: cDNA

(3) SEQUENCE DESCRIPTION: SEQ ID NO. 5:

ATGTGGCTGC CTTGGGCTCT GTTGCTTCTC TGGGTCCCAG GATGTTTTGC	50
TCTGAGCAAA TGCAGGACCG TGGCGGGCCC GTGGGGATCC CTGAGTGTGC	100
AGTGTCCCTA TGAGAAGGAA CACAGGACCC TCAACAAATA CTGGTGCAGA	150
CCACCACAGA TTTTCCTATG TGACAAGATT GTGGAGACCA AAGGGTCAGC	200
AGGAAAAAGG AACGGCCGAG TGTCCATCAG GGACAGTCCT GCAAACCTCA	250
GCTTCACAGT GACCCTGGAG AATCTCACAG AGGAGGATGC AGGCACCTAC	300
TGGTGTGGGG TGGATACACC ATGGCTCCGA GACTTTCATG ATCCCGTTGT	350
CGAGGTTGAG GTGTCCGTGT TCCCGGCATC AACGTCAATG ACACCTGCAA	400
GTATCACTGC GGCCAAGACC TCAACAATCA CAACTGCATT TCCACCTGTA	450
TCATCCACTA CCCTGTTTTGC AGTGGGTGCC ACCCACAGTG CCAGCATCCA	500
GGAGGAAACT GAGGAGGTGG TGAACTCACA GCTCCCGCTG CTCCTCTCCC	550
TGCTGGCATT GTTGCTGCTT CTGTTGGTGG GGGCCTCCCT GCTAGCCTGG	600
AGGATGTTTC AGAAATGGAT CAAAGCTGGT GACCATTTCAG AGCTGTCCCA	650
GAACCCCAAG CAGGCTGCCA CGCAGAGTGA GCTGCACTAC GCAAATCTGG	700
AGCTGCTGAT GTGGCCTCTG CAGGAAAAGC CAGCACCACC AAGGGAGGTG	750
GAGGTGGAAT ACAGCACTGT GGCCTCCCCC AGGGAAGAAC TTCACTATGC	800
CTCGGTGGTG TTTGATTCTA ACACCAACAG GATAGCTGCT CAGAGGCCTC	850
GGGAGGAGGA ACCAGATTCA GATTACAGTG TGATAAGGAA GACA	894

## (2) INFORMATION FOR SEQUENCE ID NO. 6:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 492

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(2) MOLECULE TYPE: cDNA

(3) SEQUENCE DESCRIPTION: SEQ ID NO. 6:

```
GGATGTTTTG CTCTGAGCAA ATGCAGGACC GTGGCGGGCC CGTGGGGATC   50
CCTGAGTGTG CAGTGTCCCT ATGAGAAGGA ACACAGGACC CTCAACAAAT  100
ACTGGTGCAG ACCACCACAG ATTTTCCTAT GTGACAAGAT TGTGGAGACC  150
AAAGGGTCAG CAGGAAAAAG GAACGGCCGA GTGTCCATCA GGGACAGTCC  200
TGCAAACCTC AGCTTCACAG TGACCCTGGA GAATCTCACA GAGGAGGATG  250
CAGGCACCTA CTGGTGTGGG GTGGATACAC CATGGCTCCG AGACTTTTCAT  300
GATCCCGTTG TCGAGGTTGA GGTGTCCGTG TTCCCGGCAT CAACGTCAAT  350
GACACCTGCA AGTATCACTG CGGCCAAGAC CTCAACAATC ACAACTGCAT  400
TTCCACCTGT ATCATCCACT ACCCTGTTTG CAGTGGGTGC CACCCACAGT  450
GCCAGCATCC AGGAGGAAAC TGAGGAGGTG GTGAACTCAC AG           492
```

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/NZ 99/00003**

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>				
Int Cl <sup>6</sup> : C07K 14/735; A61K 38/17				
According to International Patent Classification (IPC) or to both national classification and IPC				
<b>B. FIELDS SEARCHED</b>				
Minimum documentation searched (classification system followed by classification symbols)				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)				
STN : Sequence Search of Sequence ID3 Keyword CMRF				
ANGIS : Sequence Search of Sequence ID3				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
P,X	International Immunology Volume 10, No: 7, B J Green et al, pages 891-899 (13 March 1998) "The CMRF-35mAb recognizes a second leukocyte membrane molecule with a domain similar to the poly Ig receptor"	1-17		
P,A		18-23		
A	Immunology Volume 79(1) (May 1993), pages 55-63 Daish A et al "Expression of the CMRF-35 antigen a new member of the immunoglobulin gene superfamily, is differentially regulated on leucocytes"	1-23		
A	Leucocyte Typing V: White Cell Differ. Antigens, Proc Int Workshop Conf, 5 <sup>th</sup> (1995), meeting Date 1993, Volume 1, 1166-1167. Editor: Schlossman, S F, Publisher: Oxford University Press "A novel member of the immunoglobulin gene superfamily recognized by the mAb CMRF-35" Starling, G et al	1-23		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex				
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"I." document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width: 50%; vertical-align: top;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> </td> </tr> </table>			<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"I." document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>
<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"I." document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>			
Date of the actual completion of the international search 12 April 1999		Date of mailing of the international search report <b>- 4 MAY 1999</b>		
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929		Authorized officer  <b>K LEVER</b> Telephone No.: (02) 6283 2254		

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ 99/00003

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Eur J Immunol. 1992 Volume 22, pages 1157-1163, "Molecular Cloning of a novel member of the immunoglobulin gene superfamily homologous to the polymeric immunoglobulin receptor", D G Jackson et al	1-23



## FIGURE 1

CMRF-35-H9

13	164	24	100
A	B	C	D



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Y S V I R K T \* 301.  
ATTACAGTGTGATAAGGAAGACATaggttcctgcctcggccatcggagctctcatgagccccc 1020  
aggaagtcaggacagctcccttatacclggcccaagtccttctcagcctcggccctcgaca 1080  
acagtgacccacagacagggcagctgggtttccaggccatccctctgttgcctcagcttg 1140  
attggcttcccccaggggccagcagggctggggctccggagagcagcaggaagcaccacca 1200  
gccaccagtgccctgtcagctcttccctcttggccctgtcttccctcagctctgtgtgtg 1260  
gaggacaaagcttcttccctgggtggclccaggaaagatgtggclcacgtaggttgcacc 1320  
tgcccaatagcttctgtcactccagccccatagggaagctctggcattgcttgggagctggg 1380  
gggaactgtcaggaagagtggaagagctggccaaaggaggatctgttccctgggtggag 1440  
gggacccactaagatcagatcagaagatttcctccatctcacagacagggaactgaganna 1500  
gaggggagagagaattgtctatggctccagaaatgggtggcaagtttcttggactcttta 1560  
ggttttttttatataatgaatctaaacacagtttcaaatatcttttttggggagagtaa 1620  
aaacttatctaaaccccg 1640

Figure 2/2

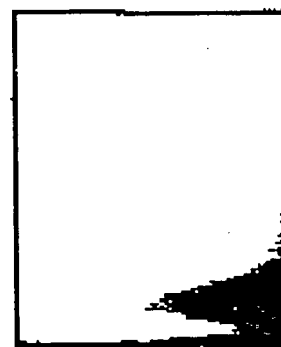
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EXPRESSION OF CMRF-35 BY L CELL TRANSFECTANTS

CMRF-35  
Transfectants



L Cells



CELL NUMBER

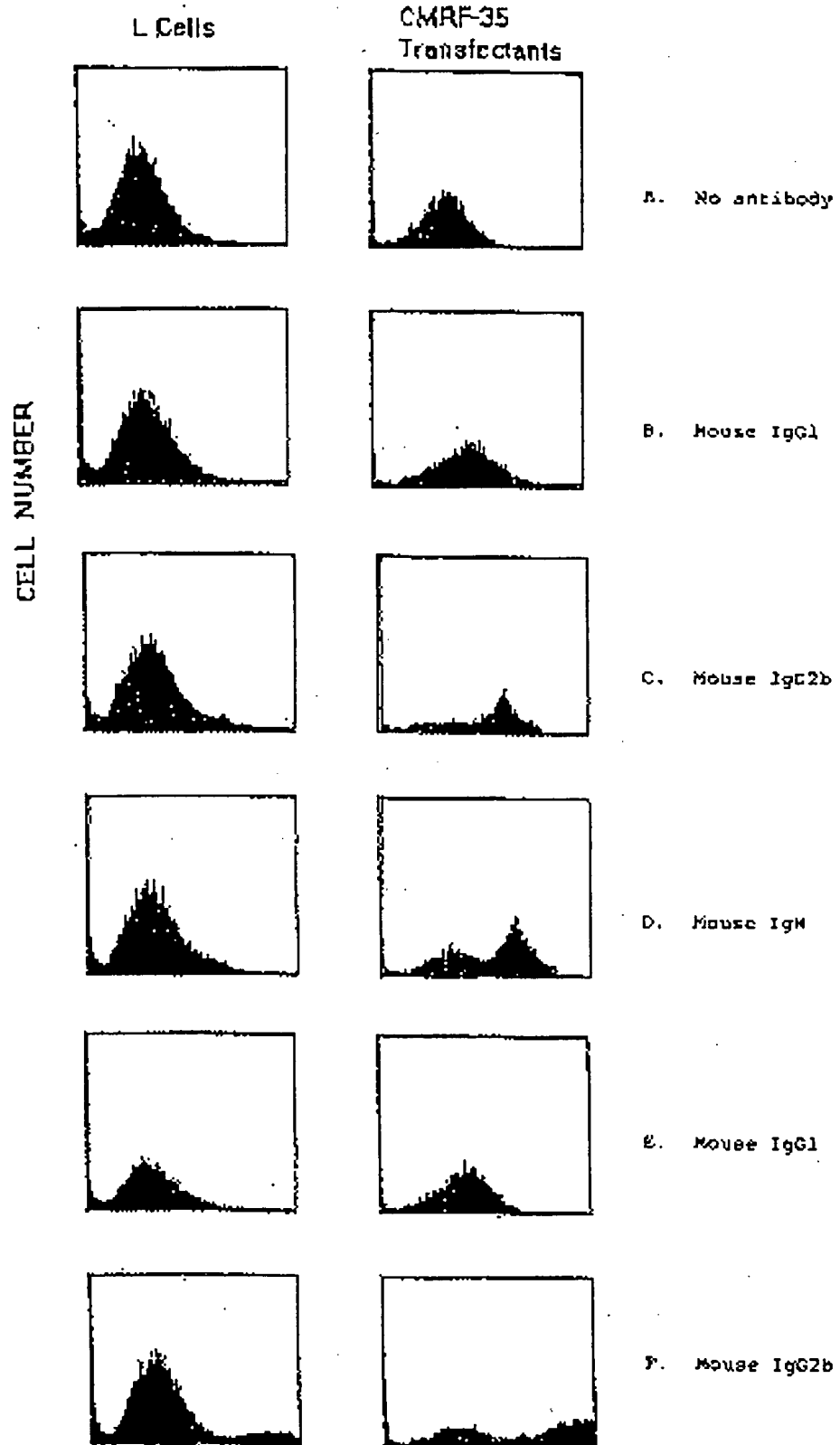
FLUORESCENCE INTENSITY

PANEL B

PANEL A

FIGURE 3

FIGURE 4 5/10



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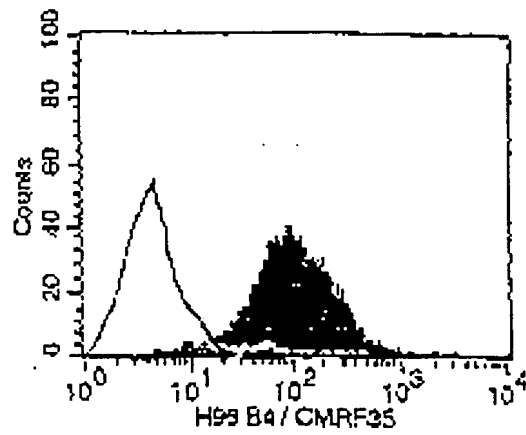


FIGURE 5



FIGURE 6a

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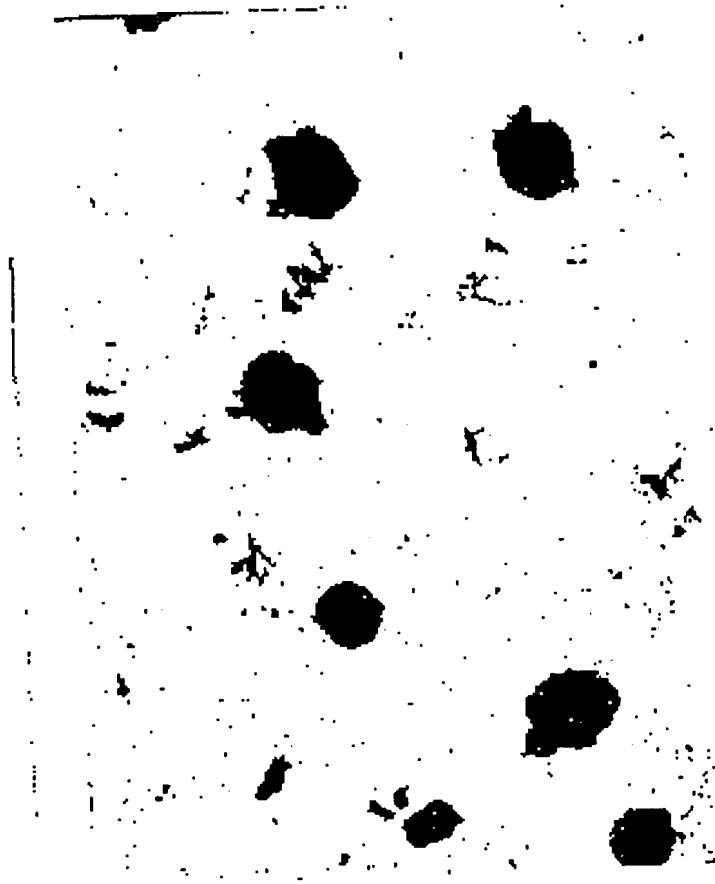


FIGURE 6b



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# Reactivity of mAb with CMRF-35-Ig Fusion Protein

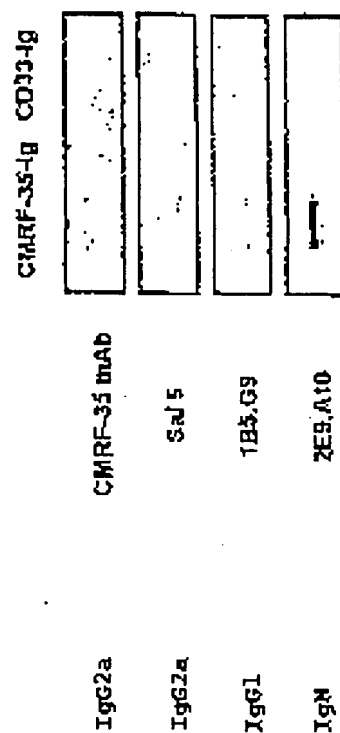


FIGURE 7

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CMRF-75

CMRF-35-H9  
CMRF-35  
Vector only  
Untransfected



Figure 8

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (1) APPLICANT: THE CORPORATION OF THE TRUSTEES  
OF THE ORDER OF THE SISTERS OF  
MERCY IN QUEENSLAND
- (2) TITLE: RECEPTOR
- (3) NUMBER OF SEQUENCES: 6
- (5) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 3.5"HD FLOPPY DISC
  - (B) COMPUTER: IBM PC COMPATIBLE
  - (C) OPERATING SYSTEM: MS-DOS
  - (D) SOFTWARE: WORD 95

## (2) INFORMATION FOR SEQUENCE ID NO. 1:

- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 301 AMINO ACIDS
  - (B) TYPE: AMINO ACID
  - (C) TOPOLOGY: LINEAR
- (2) MOLECULE TYPE: PROTEIN
- (3) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

```
MWLPWALLLI WVPGCFALSK CRTVAGPWGS LSVQCPYEKE HRTLKYNWCR   50
PPQIFLCDKI VETEGSAGKR NGRVSIRDSP ANLSFTVTLE NLTEEDAGTY  100
WCGVDTPWLR DFHDPVVEVE VSVFPASTSN TPASITAART STITTAAPPV  150
SSTTLPAVGA THSASIQEET EBVWNSQLPL LLSLLALLLL LLVGASLLAW  200
RMFQKNIEWI KAGDHSELSQ NPKQAATQSE LHYANLELLM WPLQEKAPP  250
REVEVEYSTV ASPREELHYA SVVFDSTNR LAAQRPREEE PDSYSVIRK  300
T                                                                301
```

## (2) INFORMATION FOR SEQUENCE ID NO. 2:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 298 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) TOPOLOGY: LINEAR

## (2) MOLECULE TYPE: PROTEIN

## (3) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

```

MWLPWALLLI WVPGCFALSK CRTVAGPWGS LSVQCPYEKE HRTLNKYWCR    50
PPQIFLCDKI VETKGSAGKR NGRVSIIRDSP ANLSFTVTLE NLTBEDAGTY    100
WCGVDTPWLR DFHDPVVEVE VSVFPASTSM TPASITAAKT STITTAPPPV    150
EETTLFAVGA THSASIQEET EEVNSQLPL LLSLLALLLI LLVGASLLAW    200
RNFQKWKIAG DHSLSQNPX QAATQSELHY ANLELLNWPL QEKPAFPREV    250
EVEYSTVASP REELHYASVV FDSNTNRIAA QRPREEEPDS DYSPVIRKT    298

```

## (2) INFORMATION FOR SEQUENCE ID NO. 3:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 164 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) TOPOLOGY: LINEAR

## (2) MOLECULE TYPE: PROTEIN

## (3) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

```

GCFALSKCRT VAGPWGSLSV QCPYEKEHRT LNKYWCRFPQ IFLCDKIVET    50
KGSAGKRNGR VSIRDSPANL SFTVTLENLT BEDAGTYWCG VDTPLRDFH    100
DPVVEVEVSV FPASTENTPA SITAAKTSTI TTAPPPVSST TLFAVGATHS    150
ASIQEETEEV VNSQ                                           164

```

## (2) INFORMATION FOR SEQUENCE ID NO. 4:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 903  
 (B) TYPE: NUCLEIC ACID  
 (C) STRANDEDNESS: SINGLE  
 (D) TOPOLOGY: LINEAR

## (2) MOLECULE TYPE: cDNA

## (3) SEQUENCE DESCRIPTION: SEQ ID NO. 4:

```

ATGTGGCTGC CTTGGGCTCT GTTGCTTCTC TGGGTCCCAG GATGTTTTGC 50
TCTGAGCAAA TGCAGGACCG TGGCGGGCCC GTGGGGATCC CTGAGTGTGC 100
AGTGTCCCTA TGAGAAGGAA CACAGGACCC TCAACAAATA CTGGTGCAGA 150
CCACCACAGA TTTTCCTATG TGACAAGATT GTGGAGACCA AAGGGTCAGC 200
AGGAAAAGG AACGGCCGAG TGTCCATCAG GGACAGTCCT GCAAACCTCA 250
GCTTCACAGT GACCCCTGGAG AATCTCACAG AGGAGGATGC AGGCACCTAC 300
TGGTGTGGGG TGGATACACC ATGGCTCCGA GACTTTCATG ATCCCGTTGT 350
CGAGGTTGAG GTGTCCGTGT TCCCGGCATC AACGTCAATG ACACCTGCAA 400
GTATCACTGC GSCCAAGACC TCAACAATCA CAACTGCATT TCCACCTSTA 450
TCATCCACTA CCCTGTTTGC AGTGGGTGCC ACCCACAGTG CCAGCATCCA 500
GGAGGAAACT GAGGAGGTGG TGAAGTCACA GCTCCCGCTG CTCCTCTCCC 550
TGCTGGCATT GTTCTGTCTT CTGTTGGTGG GGGCCTCCCT GCTAGCCTGG 600
AGGATGTTTC AGAAATGGAT CAAATGGATC AAGCTGGTG ACCATTGAGA 650
GCTGTCCCAG AACCCCAAGC AGGCTGCCAC GCAGAGTGAG CTGCACTACG 700
CAAATCTGGA GCTGCTGATG TGGCCTCTGC AGGAAAAGCC AGCACCACCA 750
AGGGAGGTGG AGGTGGAATA CAGCACTGTG GCCTCCCCCA GGGAAGAACT 800
TCACTATGCC TCGGTGGTGT TTGATTCTAA CACCAACAGG ATAGCTGCTC 850
AGAGGCCTCG GGAGGAGGAA CCAGATTGAG ATTACAGTGT GATAAGGAAG 900
ACA 903

```

## (2) INFORMATION FOR SEQUENCE ID NO. 5:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 894

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(2) MOLECULE TYPE: cDNA

(3) SEQUENCE DESCRIPTION: SEQ ID NO. 5:

ATGTGGCTGC	CTTGGGCTCT	GTTGCTTCTC	TGGGTCCCAG	GATGTTTTGC	50
TCTGAGCAAA	TGCAGGACCG	TGGCGGGCCC	GTGGGGATCC	CTGAGTGTGC	100
AGTGTCCCTA	TGAGAAGGAA	CACAGGACCC	TCAACAAATA	CTGGTGCAGA	150
CCACCACAGA	TTTTCTATG	TGACAAGATT	GTEGAGACCA	AAGGGTCAGC	200
AGGAAAAAGG	AACGGCCGAG	TGTCCATCAG	GCACAGTCTT	GCAAACCTCA	250
GCTTCACAGT	GACCCCTGGAG	AATCTCACAG	AGGAGGATGC	AGGCACCTAC	300
TGGTGTGGGG	TGGATACACC	ATGGCTCCGA	GACTTTCATG	ATCCCGTTGT	350
CGAGGTTGAG	GTGTCCGTGT	TCCCGGCATC	AACGTCAATG	ACACCTGCAA	400
GTATCACTGC	GGCCAAGACC	TCAACAATCA	CAACTGCATT	TCCACCTGTA	450
TCATCCACTA	CCCTTTTTGC	AGTGGGTGCC	ACCCACAGTG	CCAGCATCCA	500
GGAGGAAACT	GAGGAGGTGG	TGAACTCACA	GCTCCCGCTG	CTCCTCTCCC	550
TGCTGGCATT	GTTGCTGCTT	CTGTTGGTGG	GGGCTCCCT	GCTAGCCTGG	600
AGGATGTTTC	AGAAATGGAT	CAAAGCTGGT	GACCATTGAG	AGCTGTCCCA	650
GAACCCCAAG	CAGGCTGCCA	CGCAGAGTGA	GCTGCACTAC	GCAAATCTGG	700
AGCTGCTGAT	GTGGCCTCTG	CAGGAAAAGC	CAGCACCACC	AAGGGAGGTG	750
GAGGTGGAAT	ACAGCACTGT	GGCCTCCCCC	AGGGAAGAAC	TTCACCTATGC	800
CTCGGTGGTG	TTTGATTCTA	ACACCAACAG	GATAGCTGCT	CAGAGGCCTC	850
GGGAGGAGGA	ACCAGATTCA	GATTACAGTG	TGATAAGGAA	GACA	894

## (2) INFORMATION FOR SEQUENCE ID NO. 6:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 492

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

## (2) MOLECULE TYPE: cDNA

## (3) SEQUENCE DESCRIPTION: SEQ ID NO. 6:

```
GGATGTTTTG CTCTGAGCAA ATGCAGGACC GTGGCGGGCC CGTGGGGATC   50
CCTGAGTGTG CAGTGTCCCT ATGAGAAGGA ACACAGGACC CTCACCAAAT  100
ACTGGTGCAG ACCACCACAG ATTTTCCTAT GTGACAAGAT TGTGGAGACC  150
AAGGGGTCAG CAGGAAAAAG GAACGGCCGA GTGTCCATCA GGGACAGTCC  200
TGCACACCTC AGCTTCACAG TGACCCCTGGA GAATCTCACA GAGGAGGATG  250
CAGGCACCTA CTGGTGTGGG GTGGATACAC CATGGCTCCG AGACTTTCAT  300
GATCCCGTTG TCGAGGTTGA GGTGTCCGTG TTCCCGGCAT CAACGTCAAT  350
GACACCTGCA AGTATCACTG CGGCCAAGAC CTCAACAATC ACAACTGCAT  400
TTCCACCTGT ATCATCCACT ACCCTGPTTG CAGTGGGTGC CACCCACAGT  450
GCCAGCATCC AGGAGGAAAC TGAGGAGGTG GTGAACTCAC AG          492
```